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Running title: Ageing and the mucosal immune system

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Abstract

Bacterial and viral infections of the gastrointestinal tract are more common in the elderly and represent a major cause of morbidity and mortality. The mucosal immune system provides the first line of defence against pathogens acquired by ingestion and inhalation, but its function is adversely affected in the elderly. This aging-related decline in the immune function is termed *immunosenescence* and is associated with diminished abilities to generate protective immunity, reduced vaccine efficacy, increased incidence of cancer, inflammation and autoimmunity, and the impaired ability to generate tolerance to harmless antigens. In this review we describe our current understanding of the effects immunosenescence has on the innate and adaptive arms of the mucosal immune system in the intestine. Current estimates suggest that by the year 2050 up to 40% of the UK population will be over 65 years old, bringing with it important health challenges. A thorough understanding of the mechanisms that contribute to the development of immunosenescence is therefore crucial to help identify novel approaches to improve mucosal immunity in the elderly.

Introduction

The mucosal immune system of the gastrointestinal tract provides the first line of defence against pathogens acquired by ingestion. In addition to pathogenic organisms, the gastrointestinal tract is continuously exposed to large amounts of commensal microorganisms and food proteins. The mucosal immune system must discriminate between these harmless antigens and generate tolerance towards them, whilst retaining the ability to generate protective immune responses against pathogens. Both responses are generated in the gut-associated lymphoid tissues (GALT) comprising chiefly of the appendix, tonsils, Peyer's patches, colonic patches, caecal patches and isolated lymphoid follicles (ILF). In addition, immune cells and lymph draining from the intestine accumulates in the mesenteric lymph nodes (MLN). The highly organized microarchitecture of the GALT enables antigen, antigen presenting cells and rare antigen-specific lymphocytes to interact and mount effective immune responses. These lymphoid structures, together with the diffusely distributed effector cells within the epithelium, the underlying lamina propria, protect the intestine against infection from pathogens and their toxins (Mowat 2003).

The mucosal immune response in the intestine is significantly compromised in the elderly (termed *immunosencence*). This decline with age affects both innate and adaptive immunity and is associated with diminished antigen-specific antibody titres in the intestinal lumen and a decreased ability to generate tolerance to harmless antigens (Schmucker et al. 2003; Koga et al. 2000; Kato et al. 2003; Fujihashi and McGhee 2004; Dunn-Walters et al. 2003). This effect coincides with age-related increases in the incidence and severity of gastrointestinal infections, tumours and

inflammatory diseases, coupled with decreases in the efficacy of vaccinations (Fagiolo et al. 1993; Fujihashi et al. 2000; Fujihashi and McGhee 2004). Although many studies have addressed the age-related changes to systemic immune responses, particularly the effects of aging on T cell responses (which have been reviewed elsewhere (Akbar and Henson 2011; Henson and Akbar 2010)), much less is comparatively known of the mechanisms underlying the decline in intestinal immune function. Age-related involution of Peyer's patches is frequently discussed in the literature (for example (St. Rose et al. 2006; Fujihashi and Kiyono 2009)) but these tissues persist throughout the lifespan of many species (Liebler-Tenorio and Pabst 2006; Zidan and Pabst 2008). Furthermore, the number of ILF, individual B-cell follicles found throughout the intestines, significantly increases in the intestines of aged mice (McDonald et al. 2011).

The UK elderly population continues to expand due to a combination of factors such as increasing life expectancy and reduced birth rates. At the time of writing data suggested that ~10 million people in the UK were ≥65 years old, and this number is predicted to increase to ~15.5 million by 2030, and double to ~19 million by 2050 (Commons 2013). This significant demographic change brings with it important health challenges. Morbidity and mortality from gastrointestinal tract pathogens are substantially increased in the elderly, and the treatment of aging-related conditions absorbs the largest and growing share of the UK health care budget. A thorough understanding of the mechanisms that lead to mucosal immunosenescence is therefore crucial for the development of vaccines and therapeutic strategies to enhance mucosal immunity in the elderly.

The induction and maintenance of an antigen-specific mucosal immune response in the intestine is considered to be a multistep process occurring via the following key stages:

Step 1: Transport of gut lumenal antigen across the intestinal epithelium

Step 2: Antigen presentation by mononuclear phagocytes to T cells within the GALT and MLN

Step 3: Polarization of T cells along effector or tolerance pathways

Step 4: Antibody isotype switching, differentiation and subsequent migration of antigen-specific IgA^+ B cell immunoblasts to the intestinal lamina propria

Step 5: Local IgA production by plasma cells in the lamina propria

Step 6: Transport of IgA across the intestinal epithelium and secretion into the lumen

With the above steps in mind, this review summarizes our current understanding of how the function of the mucosal immune system in the intestine is affected by aging, particularly the effects on the innate immune system and IgA production by B cells.

Effects on the transport of lumen antigen across the intestinal epithelium

M cells: The lumen of the mammalian lower intestine contains an enormous burden of commensal microorganisms which is estimated to comprise ~10^{12} bacteria/g of contents (Whitman et al. 1998). Access of these microorganisms to the underlying host tissues is restricted by a single layer of intestinal epithelial cells bound by tight-junctions. However, to initiate a mucosal immune response against potential pathogens, antigens must first be acquired from the gut lumen. The epithelium overlying the B-cell follicles of the GALT, termed the follicle-associated epithelium

(FAE), is specialized to enable the transportation of antigen across the intestinal epithelium into the GALT (a process termed, *transcytosis*). Under steady-state conditions ~10 % of the cells within FAE are M cells (Lorenz and Newberry 2004; Kanaya et al. 2012; Tahoun et al. 2012). Villi typically lack significant numbers of M cells, but studies suggest their density may increase in response to certain inflammatory stimuli (Jang et al. 2004; Terahara et al. 2008). M cells in the FAE, in contrast to the neighbouring enterocytes, have a reduced glycocalyx, irregular brush border and reduced microvilli and are specialized for the transcytosis of particulate lumenal antigen and microorganisms across the FAE (Krahenbuhl and Neutra 2000; Knoop et al. 2009; Nakato et al. 2009; Hase et al. 2009; Nakato et al. 2012; Sato et al. 2013; Kim et al. 2011). The transcytosed antigens exit into the basolateral pocket beneath the M-cell enabling the lumenal antigens to be sampled and processed by lymphocytes and mononuclear phagocytes (MNP) (Lelouard et al. 2012; Wang et al. 2011). In the absence of M cells, antigen-specific T-cell responses in the Peyer's patches of mice orally-infected with *Salmonella enterica* serovar Typhimurium are reduced (Hase et al. 2009; Kanaya et al. 2012). This indicates that antigen transcytosis by M cells is an important initial step in the induction of some mucosal immune responses (Lelouard et al. 2012; Wang et al. 2011; Hase et al. 2009; Kanaya et al. 2012). However, some pathogens have evolved to exploit M cells to invade host tissues from the gut lumen (Wolf et al. 1981; Amerongen et al. 1991; Fujimura et al. 2004; Takahashi et al. 2008; Westphal et al. 2008; Donaldson et al. 2012; Tahoun et al. 2012).

Using an immunosenescent mouse model (≥ 18 months old C57BL/6 mice) we have shown that there is a dramatic decline in the density of mature M cells in the Peyer's

patches of aged mice which impeded the transcytosis of particulate antigen across the FAE (Kobayashi et al. 2013) (**Fig. 1**). To gain an understanding of the mechanism underlying this aging-related decline, we also examined effects on the factors which control the induction and M-cell differentiation and maturation. The production of receptor activator of NF-κB ligand (RANKL) by the network of stromal cells beneath the FAE stimulates the differentiation of receptor activator of NF-κB (RANK)-expressing enterocytes into M cells (Knoop et al. 2009). The subsequent functional maturation of M cells is then mediated by their intrinsic expression of the ETS (E26 transformation-specific) family transcription factor Spi-B (Kanaya et al. 2012). Our data show that RANKL expression by the underlying stromal cells beneath the FAE, or the initial induction of M cell differentiation, were unaffected in aged mice. Instead, specific impairments in the expression of Spi-B and the down-stream functional development of immature M cells into functionally mature M cells were observed in aged mice.

In the GALT, the chemokine CCL20 is specifically expressed by the FAE where it mediates the chemoattraction of CCR6-expressing lymphocytes and leukocytes towards the intestinal epithelium. CCL20-CCR6-stimulation has also been suggested to influence M-cell maturation as their density is reduced in CCR6-deficient mice (Westphal et al. 2008; Ebisawa et al. 2011; Kobayashi et al. 2013). The expression of CCL20 by the FAE was reduced in aged mice, consistent with data from an independent study on ILF in aged mice (McDonald et al. 2011). As a consequence of the reduced CCL20 expression, the chemoattraction of certain populations of MNP and lymphocytes, including those with apparent “M-cell-

inducing" potential (Ebisawa et al. 2011), towards the FAE were similarly reduced (Kobayashi et al. 2013).

These data suggest that the effects of aging on Spi-B and CCL20 expression appear to dramatically impede the functional maturation of M cells. Antigen-specific mucosal immune responses are markedly diminished in Spi-B-deficient (Kanaya et al. 2012) and CCR6-deficient mice (Cook et al. 2000). The density of mature M cells in the FAE of these mouse strains is also dramatically reduced (Kanaya et al. 2012; Ebisawa et al. 2011), suggesting that the effects of aging on M cell status may likewise contribute to the impaired Ag-specific mucosal immune responses observed in aged mice. These data also imply that the efficacy of M-cell-targeted vaccines may be significantly reduced in the elderly (Nochi et al. 2007; Misumi et al. 2009). However, despite this apparent deficiency, it is plausible that the effects of ageing on M cell maturation may reduce susceptibility to some pathogens that appear to exploit M cells to cross the gut epithelium and infect the host (Amerongen et al. 1991; Jones et al. 1994; Neutra et al. 1996; Sansonetti and Phalipon 1999; Donaldson et al. 2012). Susceptibility to oral prion infection is reduced in aged mice (Brown et al. 2009). Since M cells and Peyer's patches are important sites of prion uptake from the gut lumen after oral exposure (Donaldson et al. 2012; Glaysher and Mabbott 2007), the reduced density of M cells in the FAE of aged mice (Kobayashi et al. 2013) may potentially impede their ability to initially cross the gut epithelium and infect the host.

Whether similar effects of aging on M cells are observed in ILF or the multi-follicular patch-like structures in the large intestine remains to be determined. However, the

demonstration that CCL20 expression by the FAE overlying ILF is reduced in aged mice (McDonald et al. 2011) suggests M-cell density may likewise be impeded aged ILF. A thorough analysis of the aging-related factors that underpin the dramatic decline in the functional maturation of M cells will help identify novel approaches to stimulate M cell differentiation and improve mucosal immunity in the elderly.

Goblet cells: Goblet cells are characteristically considered to act as mucin secreting cells in the gut epithelium. However, in the steady-state goblet cells may also provide passages for the delivery of low molecular weight soluble antigens to $CD103^+$ MNP in the lamina propria (McDole et al. 2012). The precise immunological consequences of antigen sampling via these goblet-cell-associated antigen passages (GAPs) are uncertain, but the apparent preferential delivery of antigens to MNP with tolerogenic properties suggests an important role in regulating intestinal immune homeostasis. Goblet cell dysfunction has been associated with the development of intestinal inflammation (Dvorak and Dickersin 1980; van der Sluis et al. 2006), but whether aging influences their density, function and/or antigen sampling via GAPs is uncertain. Increased goblet cell density has been reported in the intestines of 12 month old rats (Aller et al. 2006), but no differences were observed in the FAE of aged mice (Kobayashi et al. 2013) (**Fig. 1**).

Epithelial cell barrier integrity: An intact intestinal epithelium provides an important protective barrier between the contents of the contents of the gut lumen and host tissues. Intercellular tight-junctions at the apical lateral membrane link neighbouring epithelial cells together and help determine paracellular permeability. Data from two independent studies suggest the expression of molecular components

of tight-junctions including zonula occludens-1 (ZO-1), junctional adhesion molecules (JAMs) and occludins is decreased in the intestinal epithelia of aged rats (Ren et al. 2013) and baboons (Tran and Greenwood-Van Meerveld 2013). These studies suggest that aging is associated with significant intestinal barrier dysfunction. The precise implications for mucosal immunity are uncertain. Disruption to tight junctions in the intestinal epithelium may lead to increased paracellular permeability to lumenal antigens and/or proinflammatory stimuli such as bacterial endotoxins. Whether this apparent dysregulation in intestinal permeability contributes to the low-level chronic inflammation often observed in the elderly (termed *inflamm-aging*) remains to be determined (Franceschi et al. 2000).

Effects on MNP

MNP comprise a heterogeneous population of macrophages and classical dendritic cells (Mabbott et al. 2010; Bradford et al. 2011; Hume et al. 2013) and are abundant throughout the intestine, residing within the villous cores and in distinct anatomical locations within the GALT. At these sites MNP are strategically positioned to aid the clearance of potential pathogens by phagocytosing and destroying them, or processing and presenting antigens on their surfaces to stimulate lymphocytes. For example, the sub-epithelium dome (SED) region of the GALT beneath the FAE (**Fig. 2**) contains a dense collection of MNP which readily acquires pathogens and antigens after transcytosis through M cells. Immunohistological data suggested $CD11c^+$ MNP were reduced in the Peyer's patches of one year old mice (Kato et al. 2003), and a significant decline in the number of macrophages in the lamina propria was also noted in the intestines of aged dogs (Kleinschmidt et al. 2007). However, our own analysis of Peyer's patches from aged mice (~18 months old) using a highly

sensitive immunohistochemical method of detection does not concur with the Kato study. Whereas the specific association of apparent M-cell-inducing $CD11c^+CD45R^+$(B220) cells (Ebisawa et al. 2011) within the FAE was significantly reduced in aged mice (coincident with the reduced expression of CCL20 (Kobayashi et al. 2013)), the density of $CD11c^+$ MNP in the SED was not adversely affected (**Fig. 3**). Whether aging affects phagocytosis or antigen presentation by mucosal MNP or their expression of MHC, co-stimulatory molecules or toll-like receptors, influencing the nature of the subsequent mucosal immune response (inflammatory vs. tolerogenic) is uncertain? One study has reported that $CD11c^+$ MNP from the MLN of 9 and 12 month old mice had a suboptimal ability to evoke an antigen-specific T-cell response after oral infection with the intracellular parasite *Encephalitozoon cuniculi* (Moretto et al. 2008). Interestingly, splenic $CD11c^+$ MNP from these animals displayed intact responses, indicating that aging may have differing effects on mucosal and systemic immune responses.

Although the incidence is rare in the steady-state, certain inflammatory stimuli appear to recruit MNP from the lamina propria to the gut epithelium where they insert their dendrites through the tight junctions between enterocytes to directly sample the luminal contents (Rescigno et al. 2001; Vallon-Eberhard et al. 2006; Niess et al. 2005; Farache et al. 2013). Other studies suggest that following challenge with non-invasive *Salmonella*, $CD11c^+CX_3CR1^+MHCII^+CD11b^-CD8\alpha^-$ MNP may even migrate into the intestinal lumen (Nicoletti et al. 2009; Argues et al. 2009). However, it is unclear whether aging impedes the ability of MNP to directly sample the lumenal contents of the gut.

Conversely the effects of aging on other immune cell populations such as T cells may indirectly affect the functions of MNP in the gut. Alternatively activated macrophages, typified by their high expression of arginase-1, chitinase 3-like 3 (Ym1), restin-like-α (FIZZ-1) and the mannose receptor, are important effector cells in the clearance of intestinal parasites and the modulation of tissue repair (Jenkins and Allen 2010). A Th2-polarized immune response dominated by expression of high levels of IL-4, IL-10, IL-13 and IL-21 is considered important for the induction of alternatively activated macrophages. Classically activated macrophages, in contrast, are stimulated by the Th1 cytokine IFN-γ and characteristically express high levels of inducible nitric oxide synthase which acts to kill intracellular pathogens via the production of reactive nitrogen intermediates from L-arginine. The elevated background levels of the pro-inflammatory cytokines IFN-γ, TNFα and IL-6 in the intestines of naïve aged mice appear to restrain the induction of a strong Th2 response following intestinal helminth infection and the differentiation of alternatively activated macrophages (Sugawara et al. 2011).

Effects on secretory IgA responses

The production of antigen-specific secretory immunoglobulin A (IgA) dominates the humoral mucosal immune response and provides an important, high-affinity, first line of defence in the intestine by preventing specific pathogens such as *Salmonella typhimurium*, *Shigella flexneri*, reovirus and pathogen-derived toxins from crossing the intestinal epithelium (Michetti et al. 1992; Lycke et al. 1999; Silvey et al. 2001; Macpherson et al. 2008; Boullier et al. 2009; Mantis et al. 2011). In addition to effects on pathogenic microorganisms, secretory IgA also helps regulate the composition of the commensal gut microbiota (Mantis et al. 2011).

A diverse B-cell repertoire is important to ensure provision of a wide range of antibodies against potential pathogens. This diversity is maintained by rearrangements in the immunoglobulin gene which produces B cells with unique immunoglobulin genes and different antigen specificities. High affinity antibodies are produced by B cells as a consequence of affinity maturation which occurs within the germinal centres of lymphoid tissues. In the intestine, immunoglobulin isotype class-switching to IgA appears to occur only in the organized lymphoid structures of the GALT such as Peyer's patches and ILF (Shikina et al. 2004; Barone et al. 2011; Knoop and Newberry 2012) which contain all the necessary cellular components required to generate IgA-committed B cells including B cell follicles with germinal centres, T cells and networks of follicular dendritic cells (FDCs). When a B-cell is activated by antigen it enters the germinal centre, proliferates and mutations are accumulated within the immunoglobulin gene (termed *hypermutation*). These mutations attempt to improve the antigen-binding affinity of the antibody. Thus, the newly encoded antibody is expressed on the B-cell surface and negative selection occurs whereby the cell undergoes apoptosis unless it receives rescue signals based on the antigen-affinity of the new antibody. If the B-cell receives the appropriate rescue signals, its immunoglobulin gene undergoes further rounds of mutation and selection, ultimately developing into an antibody secreting plasma cell or memory B cell.

There are conflicting reports in the literature over whether the overall quantity of IgA produced by the aged mucosal immune system is affected. Some data suggest aging has no effect on the amount of antibodies produced, whereas our own data

(alongside others) reveal a significant increase (**Fig. 4**) (Arranz et al. 1992; Senda et al. 1988; McDonald et al. 2011; Ebersole et al. 1985; Koga et al. 2000). However, antigen-specific IgA responses are adversely affected in the elderly and have a lower affinity (Taylor et al. 1992; Thoreux et al. 2000; Koga et al. 2000; Banerjee et al. 2002; Ademokun et al. 2010; Dunn-Walters et al. 2003). These contrasting effects suggest that the production of IgA becomes dysregulated as a consequence of aging (McDonald et al. 2011). The hypermutation rate is similar in young and old B cells, but aged cells have increased accumulations of mutations (Linder et al. 2012; Dunn-Walters et al. 2003; Koga et al. 2000; Banerjee et al. 2002). Furthermore, within the Peyer's patches of elderly humans the process of Ig gene selection within the GC is also decreased (Banerjee et al. 2002). The stromal FDC within the B cell follicles play key roles in the induction of antibody hypermutation and maintenance of B cell memory (Kosco-Vilbois 2003; Haberman and Shlomchik 2003). In the spleens from aged mice FDC status is severely compromised (Aydar et al. 2004; Brown et al. 2009; Brown et al. 2012). This defect in FDC status has been shown to result in impaired immune-complex retention, germinal centre formation and antibody production. Whether FDC in the GALT are similarly affected by aging is uncertain (Kato et al. 2003).

The IGHV-IGHD-IGHJ region of the immunoglobulin heavy chain gene is known as the CDR3 region and displays large diversity through recombination of different V, D and J segments. As a consequence, the diversity of the B-cell repertoire can be readily assessed by comparing immunoglobulin heavy chain CDR3 sequences and the level of hypermutations within them. In aged mice the intestinal IgA repertoire diversity has been shown to be increased (Linder et al. 2012) and skewed to one

more reflective of the systemic B-cell pool (McDonald et al. 2011). Some very old humans, however, display a dramatically reduced diversity in their peripheral blood B-cells which is associated with frailty (Gibson et al. 2009).

In addition to T-cell-dependent GC reactions, alternative modes of plasma cell generation have been described including T cell-independent processes and immunoglobulin isotype class-switching by peritoneal B-1 B cells in the lamina propria (Cerruti et al. 2011; Suzuki et al. 2010). Further studies are required to determine how aging specifically impacts on the induction of T-cell-dependent and T-cell-independent IgA responses, but in aged mice and elderly humans current evidence suggests most IgA-secreting plasma cells appear to be generated by T-cell help (Barone et al. 2011; Gibbons and Spencer 2011; Linder et al. 2012).

Once high-affinity IgA-committed B cells are selected they then circulate through the bloodstream and lymphatics to seed the lamina propria with plasma cell precursors which synthesize dimeric IgA for secretion through the epithelium into the gut lumen (Macpherson et al. 2008). The subsequent emigration of IgA^+ B cell plasmablasts from the Peyer's patches and their homing to the intestinal lamina propria has also been reported to be reduced in aged rodents (Schmucker et al. 2003; Thoreux et al. 2000). Similarly, adoptive transfer experiments showed that the migration of aged MLN lymphocytes to the lamina propria of young recipients was impaired (Thoreux et al. 2000). Whether the decline in aged plasmablast emigration coincides with their reduced expression of integrins is uncertain (Schmucker et al. 2003; Ogino et al. 2004). No differences in the expression of the polymeric immunoglobulin receptors on the basolateral plasma membrane of enterocytes in the aged small intestine have

been observed, implying that the secretion of IgA into the gut lumen is not affected by aging (Taylor et al. 1992; Daniels et al. 1998).

In addition to the secretion of IgA into the gut lumen to facilitate immune-exclusion, the transcytosis of secretory IgA-containing immune-complexes back across the epithelium by M cells may also help modulate the mucosal immune response (Rey et al. 2004; Kadaoui and Corthesy 2007; Rochereau et al. 2013). The transcytosed IgA-containing immune-complexes appear to be acquired by the underlying MNP and, as a consequence, are unable to disseminate beyond the MLNs. This selective delivery of potential gut pathogens to MNP by IgA may enable antigen recognition under neutralizing and non-inflammatory conditions without causing widespread tissue dissemination. The reduced functional maturation of M cells in the aged intestine suggests that the transcytosis of IgA-containing immune complexes would be impaired by aging (Kobayashi et al. 2013). Whether there is increased retention of IgA in the aged gut lumen (**Fig. 4**)(McDonald et al. 2011), for example as a consequence of the effects of aging on M-cell function, remains to be determined.

Effects on the gut microbiota

The mammalian intestine is host to a huge number and variety of indigenous bacteria. The composition of this microbiota has a profound effect on the host. In addition to providing nutritional benefit (vitamin synthesis, metabolism of dietary components etc.) these commensals help stimulate the maturation of the immune system and the effective generation of adaptive immune responses (Macpherson and Harris 2004; Bouskra et al. 2008). Disturbances to this balance have been implicated in the development of intestinal pathology. Therefore, much attention has

been focussed on understanding how aging-related effects on the intestinal microbiota may influence host immunity. Although a polyclonal IgA repertoire can develop in the absence of the gut microbiota, the CDR3 sequence repertoire of germ-free mice contains fewer hypermutations, and their intestines harbour fewer plasma cells (Linder et al. 2012). The exterior and interior surfaces of the FAE of Peyer's patches are colonized by distinct bacterial species which can also influence the development and maturation of the mucosal immune system. Segmented filamentous bacteria are potent activators of the mucosal immune system and preferentially attach to the exterior surface of the FAE, whereas *Alcaligenes* inhabits the interior of Peyer's patches (Obata et al. 2010). Whether the distribution of these bacteria in the GALT is significantly affected by aging is uncertain, but the gut microbiota composition of young individuals has been shown to differ significantly from that of the elderly (Biagi et al. 2010; Claesson et al. 2012).

Following weaning the human gut microbiota is considered to develop an "adult-like" composition at around two years of age (Favier et al. 2002), remaining relatively stable throughout adult life. While acute variations may occur, for example as a consequence of gastrointestinal infection, antibiotic treatment or dietary changes (David et al. 2013), this stability is dramatically reduced in old age. Data have shown that bifidobacteria significantly decrease in the elderly, whereas lactobacilli, coliforms, enterococci and *Clostridium perfringens* are increased (Hopkins et al. 2001; Mueller et al. 2006; Tiihonen et al. 2008). The precise aging-related mechanisms behind these changes to the gut microbiota are uncertain, but it is clear that they correlate significantly with effects on health status. For example, in one study suggested the microbiota of centegenarians was enriched in facultative

anaerobes which may contribute towards the increased inflammatory status in these individuals (Biagi et al. 2010). The microbial fermentation product butyrate has been shown to induce the differentiation of colonic regulatory T cells and ameliorate experimental colitis (Maslowski et al. 2009; Furusawa et al. 2013; Smith et al. 2013). Increased levels of systemic IL-6 and IL-8 in the elderly, coincided with an enrichment of Proteobacteria and a decrease in the amount of some butyrate producing bacteria (Biagi et al. 2010). Cross-talk between the host immune system and the microbiota influences the development of the mucosal immune system, which in turn regulates the microbiota (Round and Mazmanian 2009). Thus, further studies are necessary to determine whether these age-related differences in the composition of the gut microbiota are the cause of the increased inflammation in the elderly, or a consequence of the systemic inflammatory status. A detailed understanding of the mechanisms underlying host-intestinal microbiota cross-talk will help determine whether nutritional manipulation of the gut microbiota in the elderly may help preserve mucosal immunity (Schiffrin et al. 2009).

Concluding remarks

The aging-associated increase in the incidence of inflammation, autoimmunity, cancer and susceptibility to gastrointestinal infections coincides with a significant decline in host immunity and the efficacy of vaccinations. As discussed above, this immunosenescence has multiple effects both within the innate and adaptive arms of the mucosal immune system in the intestine. Despite significant progress in our understanding of the effects of aging on the mucosal immune system in the intestine, significant gaps remain. A thorough analysis of the cellular and molecular mechanisms that underpin the dramatic aging-related decline in immune

homeostasis will help identify the factors that enhance susceptibility to orally-acquired pathogens and reduce the efficacy of mucosal vaccinations, and identify novel approaches to improve mucosal immunity in the elderly.

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Figure 1 M-cell density is significantly reduced in the FAE of aged Peyer's patches. (a) Z-stack image from whole-mount image of a Peyer's patch from a young mouse showing an M-cell with characteristic basolateral pocket ($GP2^+$ cell; green) and UEA-1-binding goblet cell (red). Tissue counterstained to detect f-actin (blue). (b) M-cell density is dramatically reduced in the FAE of Peyer's patches from aged mice. Whole-mount images of Peyer's patches from each mouse group are shown. The positions of the X-Z and Y-Z projections of the FAE are indicated by the broken line in the main X-Y images. Closed arrows indicate $GP2^+$ M cells (green). Open arrow-heads indicate $GP2^-$UEA-1-binding goblet cells (red). (c) Morphometric analysis indicated that the density of M cells in the FAE of aged mice was significantly reduced. (d) Aging did not affect the density of goblet cells in the FAE. Data are derived from 3-5 Peyer's patches from at least 4 mice from each group (Kobayashi et al. 2013).

Figure 2 Mononuclear phagocytes (MNP) are abundant in the intestine. Fluorescent confocal microscopy analysis of Peyer's patches in the intestines of a Tg(Csf1r-EGFP)1Hume "MacGreen" mouse in which the Csf1r promoter drives expression of enhanced green fluorescent protein (EGFP) in all MNP populations (Sasmono et al. 2003). (a) By whole-mount imaging, abundant MNP expressing EGFP are detected in the lamina propria of villi and beneath the follicle-associated epithelium (FAE) of the Peyer's patches. (b) Analysis of tissue sections shows abundant MNP in the lamina propria of villi and in the sub-epithelial dome (SED) of the Peyer's patches.

Figure 3 Effects of aging on the density of CD11c$^+$ cells in Peyer's patches. (a) IHC analysis of the distribution of CD11c$^+$ cells (red) in the Peyer's patches of young and aged mice. Morphometric analysis suggested there was no difference in the density of CD11c$^+$ cells in the sub-epithelial dome (SED) in Peyer's patches from each mouse group. (b) IHC analysis of the distribution of CD11c$^+$ (red) and CD45R$^+$ (B220, green) cells in FAE of Peyer's patches from young and aged mice. Arrows show CD11c$^+$CD45R$^+$ cells with apparent "M-cell-inducing" potential (Ebisawa et al. 2011). Morphometric analysis suggested the density of these CD11c$^+$CD45R$^+$ cells was significantly reduced in the FAE of aged mice. Data are derived from 3-5 Peyer's patches from at least 4 mice from each group (Kobayashi et al. 2013).

Figure 4 Total faecal IgA levels are elevated in aged mice. The concentration of total IgA in the faeces of aged mice is significantly elevated when compared to young mice (McDonald et al. 2011). Figure shows authors' own data from the analysis of individual faecal pellets from at least 4 mice from each group.

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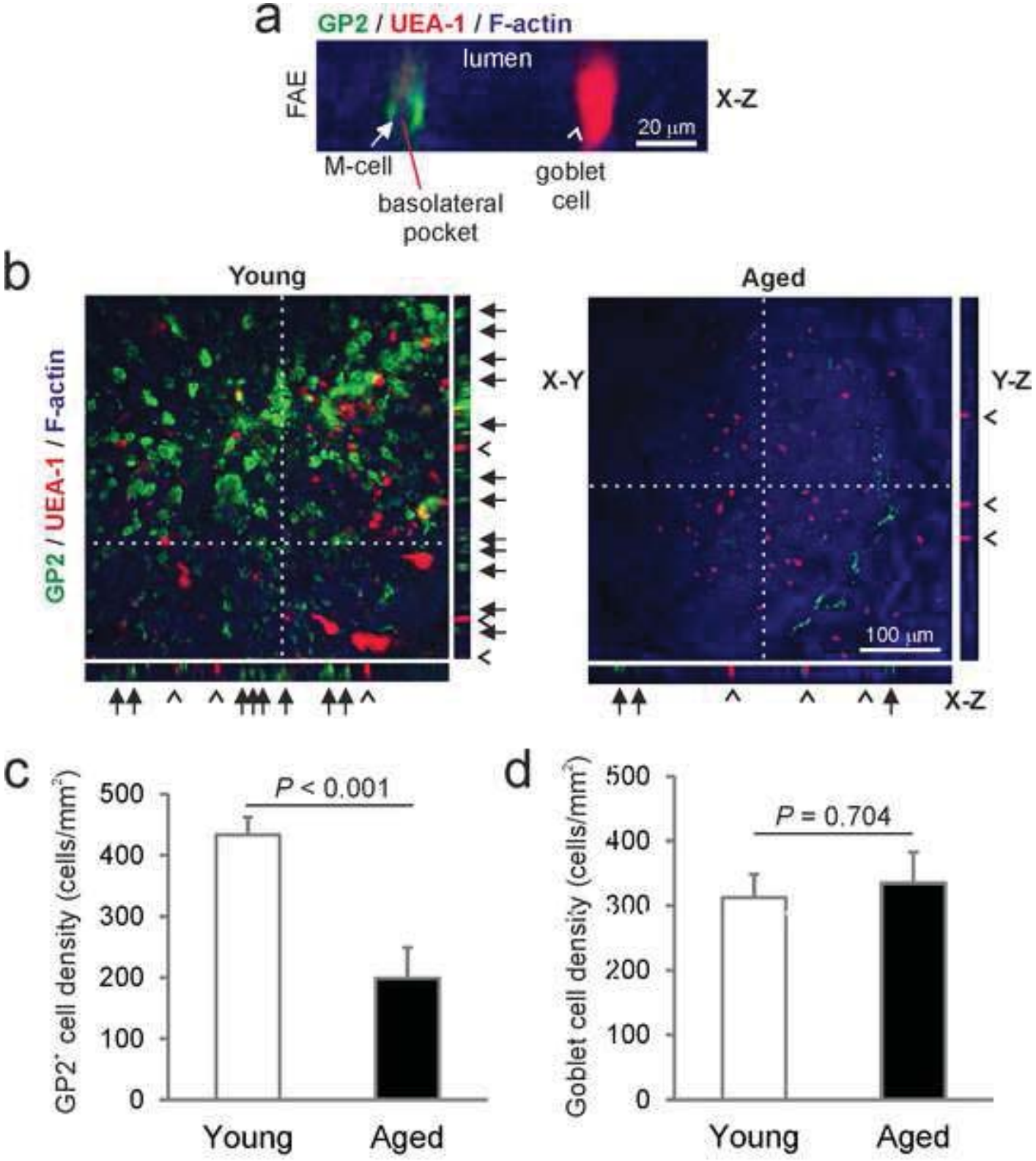

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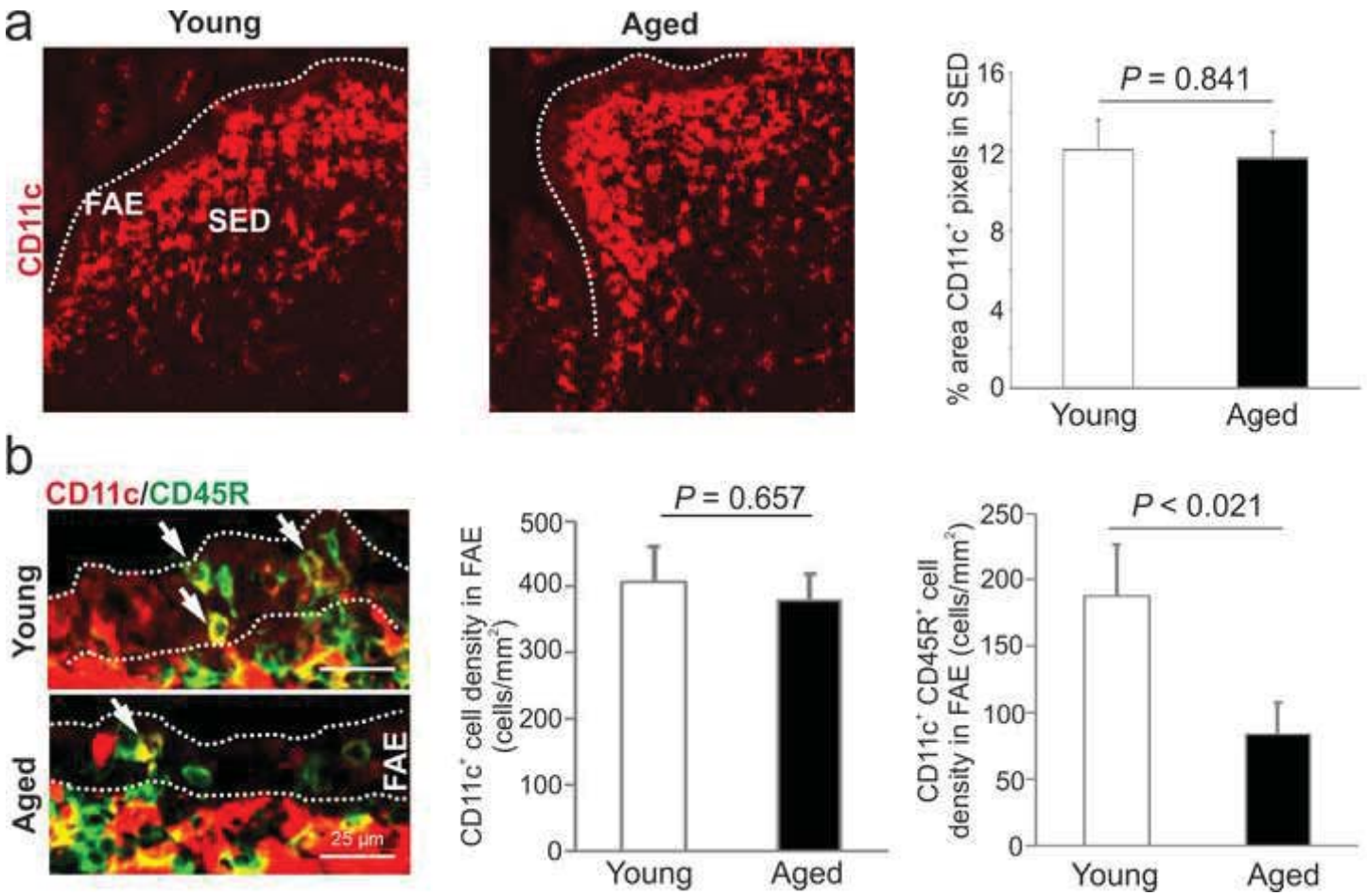

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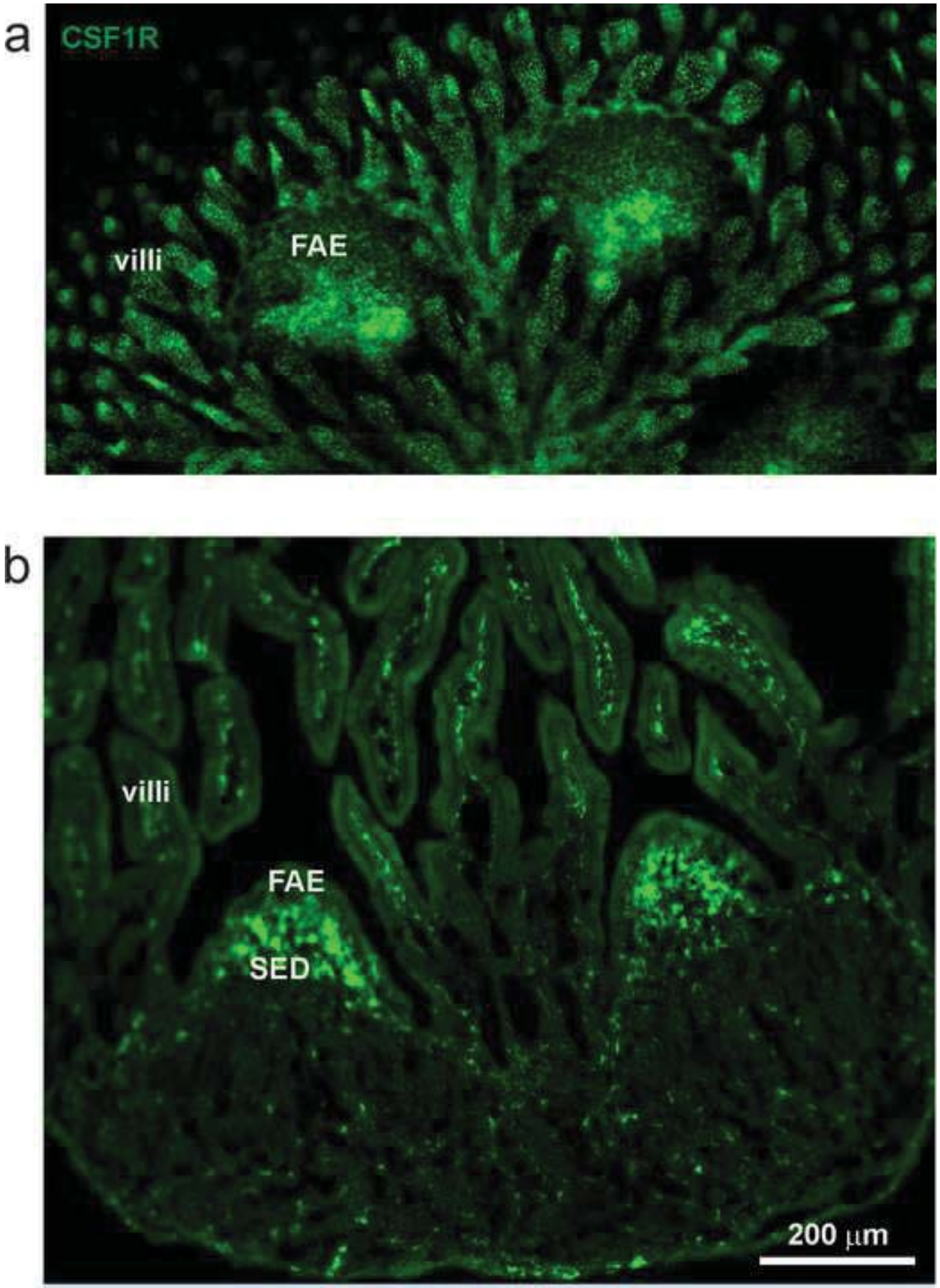

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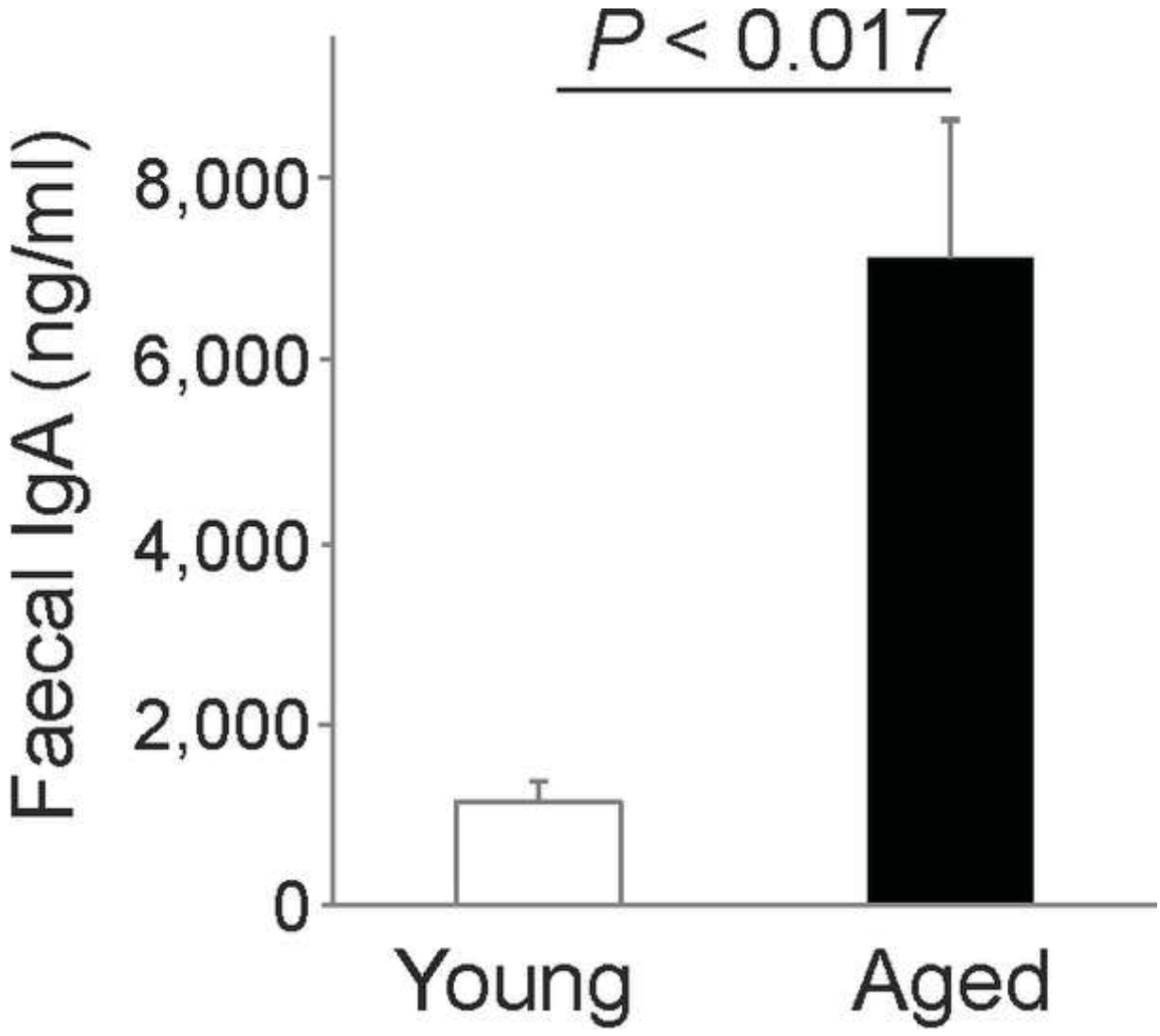

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